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(54) Title: **BRANCHED MOLECULAR SCAFFOLDS FOR LINKING POLYMER RESIDUES TO BIOLOGICALLY ACTIVE MOIETIES**

(57) Abstract: Branched molecular scaffolds are provided which are capable of linking two polymer residues (derived, for example, from polyethylene glycol) to two, three or four residues derived from biologically active molecules (e.g. from whole antibodies or from functionally active fragments or derivatives thereof), the latter being attached to the scaffold by means of hydrolytically stable linkages.



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BRANCHED MOLECULAR SCAFFOLDS FOR LINKING POLYMER RESIDUES TO BIOLOGICALLY ACTIVE MOIETIES

The present invention relates to branched molecular scaffolds which are capable
5 of linking two polymer residues (derived, for example, from polyethylene glycol) to two,
three or four residues derived from biologically active molecules. Methods for the
production of such molecules, and pharmaceutical compositions containing them, are also
provided.

The hydrophilic polymer polyethylene glycol (PEG) has been covalently attached
10 to biologically active molecules for a variety of reasons, including to increase water
solubility (Greenwald *et al.*, *J. Org. Chem.*, 1995, **60**, 331-336), to extend the circulating
half-life and to reduce immunogenicity (Chapman, *Advanced Drug Delivery Reviews*,
2002, **54**, 531-545). In general, site-specific attachment of PEG molecules is preferable
to random attachment, which can adversely affect the biological activity of the molecule.

15 In order to attach a high enough molecular weight of PEG to the biologically
active molecule, branched PEG molecules have been used in place of very high molecular
weight linear polymer chains which can be difficult to prepare, as well as being
expensive.

For various reasons, it is considered advantageous to attach more than one
20 biologically active molecule to a single branched PEG scaffold. In such instances, for
example, a substantial increase in potency can be observed.

Branched PEG polymers comprising a single site for the attachment of one or
more biologically active molecules are described, for example, in US patents 6,362,254
and 5,932,462.

25 US 6,251,382 provides a branched scaffold comprising *inter alia* a multiplicity of
separate sites for attachment of at least two polymer chains and at least two biologically
active molecules. The molecules described therein are stated to be biodegradable,
polymer-based conjugates having the following formula:



wherein

(m) and (n) independently represent positive integers, preferably from about 1 to about 6 each;

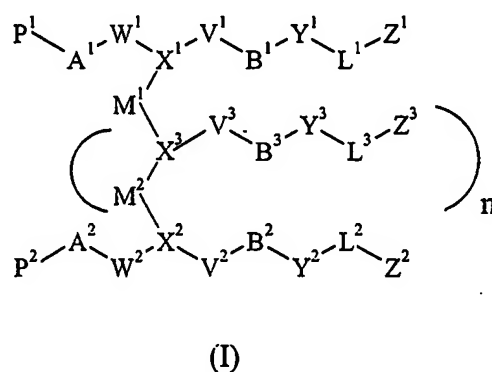
D is a residue of a biologically active moiety;

M is a multifunctional linker/spacer moiety; and

5 R_1 is a polymer residue.

The present invention provides new branched molecular scaffolds which are capable of linking two polymer residues (derived, for example, from PEG) to two, three or four residues derived from biologically active molecules, the latter being attached to the scaffold by means of hydrolytically stable linkages.

10 Thus, the present invention provides a compound of formula (I):



wherein

- 15 P^1 and P^2 independently represent a polymer residue;
 Z^1 , Z^2 and Z^3 independently represent the residue of a biologically active moiety;
 X^1 , X^2 and X^3 independently represent CR^1 or N;
 A^1 and A^2 independently represent $-CONH-$, $-NHCO-$, $-OC(O)N(R^2)-$,
 $-N(R^2)C(O)O-$ or $-NHCONH-$;
20 B^1 , B^2 and B^3 independently represent $-CONH-$ or $-CO-$;
 V^1 and V^2 independently represent a covalent bond or $-(CH_2)_v-$;
 W^1 and W^2 independently represent a covalent bond or $-(CH_2)_w-$;
 Y^1 , Y^2 and Y^3 independently represent $-(CH_2)_y-$;
 L^1 , L^2 and L^3 independently represent a spacer group;
25 M^1 and M^2 independently represent a covalent bond or $-(CH_2)_m-$;
 R^1 represents hydrogen or C_{1-4} alkyl;
 R^2 represents hydrogen or C_{1-4} alkyl;

n is zero, 1 or 2;

v is 1, 2, 3 or 4;

w is 1, 2, 3 or 4;

y is 1, 2, 3, 4, 5 or 6; and

5 m is 1, 2 or 3.

The present invention falls within the broadest generic scope of US 6,251,382.

However, there is no specific disclosure therein of a compound falling within the scope of formula (I) as depicted above.

As used herein, the term "C₁₋₄ alkyl" refers to straight-chained and branched alkyl
10 groups containing 1 to 4 carbon atoms. Such groups are methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *sec*-butyl and *tert*-butyl.

As used herein, the term "residue" will be understood to mean that portion of a polymer or of a biologically active moiety which remains after it has undergone a substitution reaction as such terminology is familiar to the person skilled in the art.

15 The polymer residues P¹ and P² in the compounds of formula (I) above will suitably be residues of substantially water-soluble, substantially non-antigenic polymers as described, for example, in US 6,251,382 B1, with particular reference to the passage running from column 16, line 52 to column 18, line 14, the contents of which are herein incorporated by reference. Typical polymers of which P¹ and P² are residues include
20 polyalkylene oxides such as polyethylene glycols (PEGs). As regards attaching PEG moieties in general, reference is made to "Poly(ethyleneglycol) Chemistry, Biotechnical and Biomedical Applications", 1992, J. Milton Harris (ed), Plenum Press, New York; "Poly(ethyleneglycol) Chemistry and Biological Applications", 1997, J. Milton Harris and S. Zalipsky (eds), American Chemical Society, Washington DC; and "Bioconjugation
25 Protein Coupling Techniques for the Biomedical Sciences", 1998, M. Aslam and A. Dent, Grove Publishers, New York. Particular PEG molecules include 20K methoxy-PEG-amine (obtainable from Nektar, formerly Shearwater; Rapp Polymere; and SunBio) and M-PEG-SPA (obtainable from Nektar, formerly Shearwater).

Suitably, P¹ and P² are identical.

30 In the compounds of formula (I) above, the residues Z¹, Z² and Z³ will suitably be residues of the entities referred to in, for example, US 6,251,382 B1, with particular reference to the passage running from column 18, line 15 to column 22, line 67, the contents of which are herein incorporated by reference. Typical biologically active

moieties of which Z^1 , Z^2 and Z^3 are residues include antibodies and antibody fragments such as those referred to in US 6,251,382 B1, column 22, lines 14-22.

Thus, the residues Z^1 , Z^2 and Z^3 include whole antibodies and functionally active fragments or derivatives thereof and may be, but are not limited to, polyclonal,
5 monoclonal, multi-valent, multi-specific, humanized or chimeric antibodies, single chain antibodies, Fab fragments, Fab' and F(ab')₂ fragments and epitope-binding fragments of any of the above.

Antibodies include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e. molecules that contain an antigen binding site
10 that specifically binds an antigen. The immunoglobulin molecules of the invention can be of any class (e.g. IgG, IgE, IgM, IgD or IgA) or subclass of immunoglobulin molecule.

Monoclonal antibodies may be prepared by any method known in the art such as the hybridoma technique (Kohler & Milstein, *Nature*, 1975, 256, 495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today*,
15 1983, 4, 72) and the EBV-hybridoma technique (Cole *et al.*, "Monoclonal Antibodies and Cancer Therapy", pp. 77-96, Alan R. Liss, Inc., 1985).

Antibodies for use in the invention may also be generated using single lymphocyte antibody methods by cloning and expressing immunoglobulin variable region cDNAs generated from single lymphocytes selected for the production of specific antibodies by,
20 for example, the methods described by Babcook, J. *et al.*, *Proc. Natl. Acad. Sci. USA*, 1996, 93(15), 7843-7848, and in WO 92/02551.

Humanized antibodies are antibody molecules from non-human species having one or more complementarity determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule (see, for example, US
25 5,585,089).

Chimeric antibodies are those antibodies encoded by immunoglobulin genes that have been genetically engineered so that the light and heavy chain genes are composed of immunoglobulin gene segments belonging to different species. These chimeric antibodies are likely to be less antigenic. Bivalent antibodies may be made by methods known in the
30 art (Milstein *et al.*, *Nature*, 1983, 305, 537-539; WO 93/08829; Traunecker *et al.*, *EMBO J.*, 1991, 10, 3655-3659). Multi-valent antibodies may comprise multiple specificities or may be monospecific (see, for example, WO 92/22853).

The antibodies for use in the present invention can also be generated using various phage display methods known in the art and include those disclosed by Brinkman *et al.*, *J. Immunol. Methods*, 1995, **182**, 41-50; Ames *et al.*, *J. Immunol. Methods*, 1995, **184**, 177-186; Kettleborough *et al.* *Eur. J. Immunol.*, 1994, **24**, 952-958; Persic *et al.*, *Gene*, 1997
5 187, 9-18; and Burton *et al.*, *Advances in Immunology*, 1994, **57**, 191-280; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; and WO 95/20401; and US 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743; and 5,969,108. Techniques for the production of single chain antibodies, such as those
10 described in US 4,946,778, can also be adapted to produce single chain antibodies. Also, transgenic mice, or other organisms, including other mammals, may be used to express humanized antibodies.

Particular antibody fragments include those described in international patent applications PCT/GB2004/002810, PCT/GB2004/002870 and PCT/GB2004/002871 (all
15 filed on 1 July 2004), claiming priority variously from United Kingdom patent applications 0315450.7, 0315457.2 (both filed on 1 July 2003) and 0319588.0 (filed on 20 August 2003).

Suitably, Z^1 and Z^2 are identical.

In one embodiment, X^1 represents CR^1 . In another embodiment, X^1 represents N.
20 In one embodiment, X^2 represents CR^1 . In another embodiment, X^2 represents N.
In one embodiment, X^3 represents CR^1 . In another embodiment, X^3 represents N.
Suitably, X^1 and X^2 are identical.

Suitably, A^1 represents -CONH- or -NHCO-. In one embodiment, A^1 represents -CONH-. In another embodiment, A^1 represents -NHCO-.

25 Suitably, A^2 represents -CONH- or -NHCO-. In one embodiment, A^2 represents -CONH-. In another embodiment, A^2 represents -NHCO-.

Suitably, A^1 and A^2 are identical.

In one embodiment, B^1 represents -CONH-. In another embodiment, B^1 represents -CO-. Where B^1 represents -CONH-, X^1 typically represents CH. Where B^1
30 represents -CO-, X^1 typically represents N.

In one embodiment, B^2 represents -CONH-. In another embodiment, B^2 represents -CO-. Where B^2 represents -CONH-, X^2 typically represents CH. Where B^2 represents -CO-, X^2 typically represents N.

In one embodiment, B^3 represents $-\text{CONH}-$. In another embodiment, B^3 represents $-\text{CO}-$. Where B^3 represents $-\text{CONH}-$, X^3 typically represents CH . Where B^3 represents $-\text{CO}-$, X^3 typically represents N .

Suitably, B^1 and B^2 are identical.

5 In a preferred embodiment, V^1 represents a covalent bond. In another embodiment, V^1 represents $-(\text{CH}_2)_v-$ in which v is as defined above.

In a preferred embodiment, V^2 represents a covalent bond. In another embodiment, V^2 represents $-(\text{CH}_2)_v-$ in which v is as defined above.

10 In a preferred embodiment, V^3 represents a covalent bond. In another embodiment, V^3 represents $-(\text{CH}_2)_v-$ in which v is as defined above.

Suitably, V^1 and V^2 are identical.

In one embodiment, W^1 represents a covalent bond. In another embodiment, W^1 represents $-(\text{CH}_2)_w-$ in which w is as defined above.

15 In one embodiment, W^2 represents a covalent bond. In another embodiment, W^2 represents $-(\text{CH}_2)_w-$ in which w is as defined above.

Suitably, W^1 and W^2 are identical.

Suitably, Y^1 and Y^2 are identical.

20 The spacer groups L^1 , L^2 and L^3 will suitably comprise any moiety familiar to the person skilled in the art which is capable of forming a bridge between the alkylene chain Y^1 , Y^2 and (where present) Y^3 and the residue Z^1 , Z^2 and (where present) Z^3 respectively. For example, where Z^1 and/or Z^2 and/or Z^3 is the residue of a polypeptide molecule (e.g. an antibody or a fragment thereof) containing a cysteine residue the corresponding spacer group L^1 and/or L^2 and/or L^3 will suitably be a maleimide residue, which may be covalently linked to the cysteine-containing polypeptide residue Z^1 and/or Z^2 and/or Z^3 via a thiol linkage and to the alkylene chain Y^1 and/or Y^2 and/or Y^3 through the maleimide nitrogen atom.

Suitably, L^1 and L^2 are identical.

In one embodiment, M^1 represents a covalent bond. In another embodiment, M^1 represents $-(\text{CH}_2)_m-$ in which m is as defined above.

30 In one embodiment, M^2 represents a covalent bond. In another embodiment, M^2 represents $-(\text{CH}_2)_m-$ in which m is as defined above.

In a preferred embodiment, R^1 is hydrogen. In another embodiment, R^1 represents C_{1-4} alkyl, especially methyl.

In a preferred embodiment, R^2 is hydrogen. In another embodiment, R^2 represents C_{1-4} alkyl, especially methyl.

Suitably, n is zero or 1.

In a preferred embodiment, n is zero, in which case M^1 is directly attached to X^2 .

5 In another embodiment, n is 1. In a further embodiment, n is 2.

In one embodiment, w is 1. In another embodiment, w is 2. In an additional embodiment, w is 3. In a further embodiment, w is 4. Favourably, w is 1 or 2.

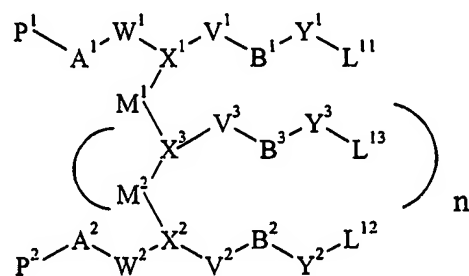
In one embodiment, y is 1. In another embodiment, y is 2. In an additional embodiment, y is 3. In a further embodiment, y is 4. In a still further embodiment, y is 5.

10 In a yet further embodiment, y is 6. Favourably, y is 2, 3 or 4, typically 2 or 4.

In one embodiment, m is 1. In another embodiment, m is 2. In an additional embodiment, m is 3. Favourably, m is 2.

In another aspect, the present invention provides novel scaffold molecules which are valuable intermediates for the attachment of the biologically active moieties of which

15 Z^1 , Z^2 and Z^3 are residues. Thus, the invention also provides a compound of formula (II):



(II)

wherein

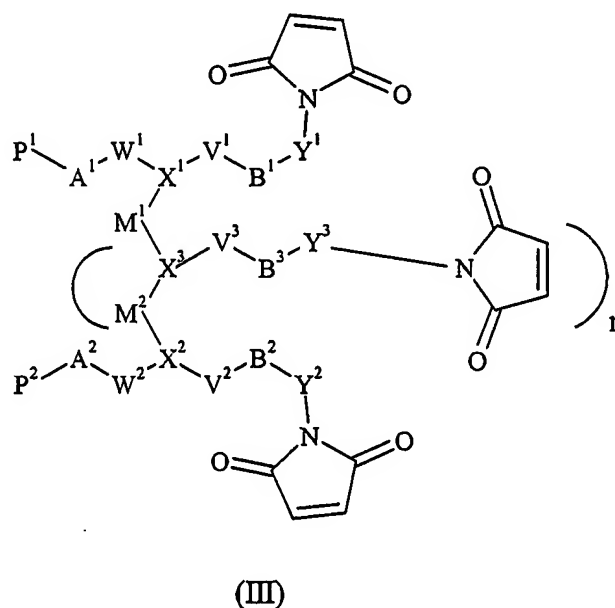
20 L^{11} , L^{12} and L^{13} represent groups capable of attaching the residue Z^1 , Z^2 and Z^3 respectively, or capable of being converted into such groups; and

each of the other variables is as defined above in relation to formula (I).

Where Z^1 and/or Z^2 and/or Z^3 is the residue of a polypeptide molecule (e.g. an antibody or a fragment thereof), the corresponding group L^1 and/or L^2 and/or L^3 may be
 25 attached to the polypeptide through any available amino acid side-chain or terminal amino acid functional group located in the antibody fragment, for example any free amino, imino, thiol, hydroxy or carboxyl group. Such amino acids may occur naturally

in, for example, the antibody fragment or may be engineered into the fragment using recombinant DNA methods (see, for example, US 5,219,996). In a preferred aspect of the invention the two groups are covalently linked through a thiol group of a cysteine residue located in the fragment. The covalent linkage will generally be a disulphide bond or a sulphur-carbon bond, preferably the latter. In one example where a thiol group is used as the point of attachment appropriately activated groups, for example thiol-selective derivatives such as maleimide and cysteine derivatives, may be used.

In a preferred feature, the groups L^{11} , L^{12} and (where present) L^{13} are identical and represent maleimide derivatives attached to the remainder of the molecule through the maleimide nitrogen atom. Accordingly, one illustrative subset of the compounds of formula (II) above is represented by the compounds of formula (III):



wherein each of the variables is as defined above in relation to formula (I).

According to a further aspect of the invention there is provided a pharmaceutical composition which comprises a compound of formula (I) as defined above in association with one or more pharmaceutically acceptable carriers, excipients or diluents.

Pharmaceutical compositions according to the invention may take a form suitable for oral, buccal, parenteral, nasal, topical, ophthalmic or rectal administration, or a form suitable for administration by inhalation or insufflation.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets, lozenges or capsules prepared by conventional means with

pharmaceutically acceptable excipients such as binding agents (e.g. pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methyl cellulose); fillers (e.g. lactose, microcrystalline cellulose or calcium hydrogenphosphate); lubricants (e.g. magnesium stearate, talc or silica); disintegrants (e.g. potato starch or sodium glycollate); or wetting agents (e.g. sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents, emulsifying agents, non-aqueous vehicles or preservatives. The preparations may also contain buffer salts, flavouring agents, colouring agents or sweetening agents, as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

The compounds of formula (I) may be formulated for parenteral administration by injection, e.g. by bolus injection or infusion. Formulations for injection may be presented in unit dosage form, e.g. in glass ampoules or multi-dose containers, e.g. glass vials. The compositions for injection may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilising, preserving and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile pyrogen-free water, before use.

In addition to the formulations described above, the compounds of formula (I) may also be formulated as a depot preparation. Such long-acting formulations may be administered by implantation or by intramuscular injection.

For nasal administration or administration by inhalation, the compounds according to the present invention may be conveniently delivered in the form of an aerosol spray presentation for pressurised packs or a nebuliser, with the use of a suitable propellant, e.g. dichlorodifluoromethane, fluorotrichloromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas or mixture of gases.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack or dispensing device may be accompanied by instructions for administration.

For topical administration the compounds according to the present invention may
5 be conveniently formulated in a suitable ointment containing the active component suspended or dissolved in one or more pharmaceutically acceptable carriers. Particular carriers include, for example, mineral oil, liquid petroleum, propylene glycol, polyoxyethylene, polyoxypropylene, emulsifying wax and water. Alternatively, the compounds according to the present invention may be formulated in a suitable lotion
10 containing the active component suspended or dissolved in one or more pharmaceutically acceptable carriers. Particular carriers include, for example, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, benzyl alcohol, 2-octyldodecanol and water.

For ophthalmic administration the compounds according to the present invention
15 may be conveniently formulated as microionized suspensions in isotonic, pH-adjusted sterile saline, either with or without a preservative such as a bactericidal or fungicidal agent, for example phenylmercuric nitrate, benzylalkonium chloride or chlorhexidine acetate. Alternatively, for ophthalmic administration compounds may be formulated in an ointment such as petrolatum.

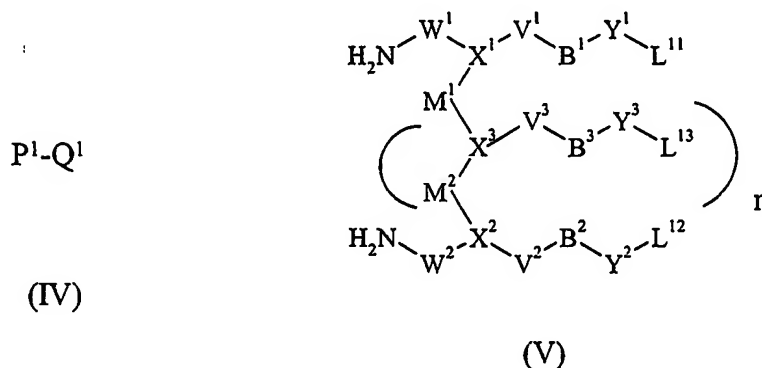
20 For rectal administration the compounds according to the present invention may be conveniently formulated as suppositories. These can be prepared by mixing the active component with a suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and so will melt in the rectum to release the active component. Such materials include, for example, cocoa butter, beeswax and polyethylene glycols.

25 The quantity of a compound of the invention required for the prophylaxis or treatment of a particular condition will vary depending on the compound chosen and the condition of the patient to be treated. In general, however, daily dosages may range from around 10 ng/kg to 1000 mg/kg, typically from 100 ng/kg to 100 mg/kg, e.g. around 0.01 mg/kg to 40 mg/kg body weight for oral or buccal administration, from around 10 ng/kg
30 to 50 mg/kg body weight for parenteral administration, and from around 0.05 mg to around 1000 mg, e.g. from around 0.5 mg to around 1000 mg, for nasal administration or administration by inhalation or insufflation.

The compounds of formula (I) may be prepared by a process which comprises attachment of the residues Z^1 , Z^2 and (where present) Z^3 to the appropriate compound of formula (II). This process may be accomplished using procedures which are well known to the person skilled in the art such as, for example, the methods described in US

5 6,251,382 B1, with particular reference to column 23, lines 1 to 50, the contents of which
are herein incorporated by reference.

The compounds of formula (II) wherein P¹ and P² are identical and A¹ and A² are both -CONH- may be prepared by a process which comprises reacting a compound of formula (IV) with a compound of formula (V):



wherein Q¹ represents an activated carboxylate moiety; and the remaining variables are as defined above.

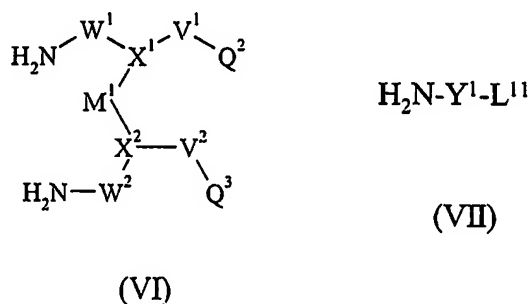
15 Examples of activated carboxylate moieties for the substituent Q¹ include acid chlorides; acid anhydrides; and the ester formed when a carboxylic acid (Q¹ = -CO₂H) is reacted with *N*-hydroxysuccinimide.

The reaction between compounds (IV) and (V) is conveniently effected in a suitable solvent, e.g. dichloromethane, typically in the presence of an organic base, e.g. triethylamine.

The compounds of formula (II) wherein P¹ and P² are identical and A¹ and A² are both -OC(O)N(H)- may be prepared by a process which comprises reacting a compound of formula P¹-OC(O)R^x, wherein R^x represents a readily displaceable group such as a halogen atom (e.g. chloro), 4-nitrophenoxy or 1-succinimidylxy; with a compound of
25 formula (V) as defined above.

The requisite intermediate of formula $P^1\text{-OC(O)R}^x$ may be prepared by treating a compound of formula $P^1\text{-OH}$ with, for example, phosgene, 4-nitrophenyl chloroformate or N,N' -disuccinimidyl carbonate.

By way of illustration, the compounds of formula (V) wherein n is zero, B^1 and B^2 are both -CONH- , Y^1 and Y^2 are identical and L^{11} and L^{12} are identical may be prepared by a process which comprises reacting a compound of formula (VI) with a compound of formula (VII):

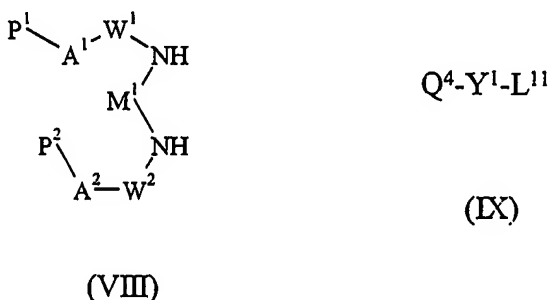


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wherein Q^2 and Q^3 independently represent an activated carboxylate moiety as defined above for Q^1 ; and the remaining variables are as defined above.

The reaction between compounds (VI) and (VII) is conveniently effected in a suitable solvent, e.g. dichloromethane, typically in the presence of an organic base, e.g. triethylamine.

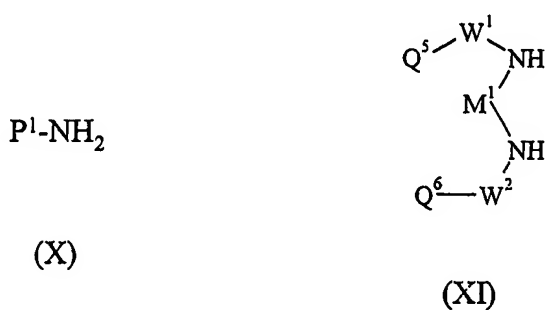
In an alternative procedure, and by way of illustration, the compounds of formula (II) wherein n is zero, X^1 and X^2 are both N , B^1 and B^2 are both -CO- , V^1 and V^2 are both covalent bonds, Y^1 and Y^2 are identical and L^{11} and L^{12} are identical may be prepared by a process which comprises reacting a compound of formula (VIII) with a compound of formula (IX):



wherein Q^4 represents an activated carboxylate moiety as defined above for Q^1 ; and the remaining variables are as defined above.

The reaction between compounds (VIII) and (IX) is conveniently effected in a suitable solvent, e.g. dichloromethane, typically in the presence of an organic base, e.g. triethylamine.

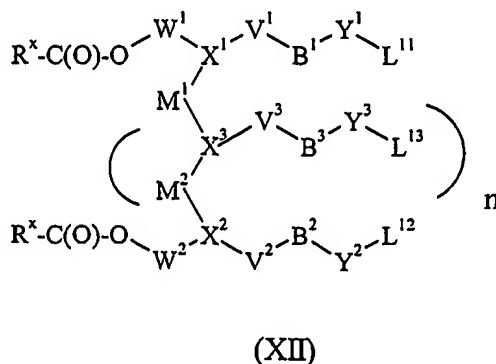
The compounds of formula (VIII) wherein P^1 and P^2 are identical and A^1 and A^2 are both $-NHCO-$ may be prepared by reacting a compound of formula (X) with a compound of formula (XI):



wherein Q^5 and Q^6 independently represent an activated carboxylate moiety as defined above for Q^1 ; and the remaining variables are as defined above.

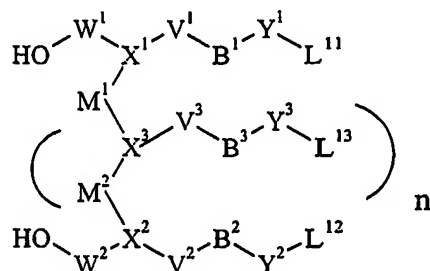
The reaction between compounds (X) and (XI) is conveniently effected in a suitable solvent, e.g. dichloromethane, typically in the presence of an organic base, e.g. triethylamine.

The compounds of formula (II) wherein P^1 and P^2 are identical and A^1 and A^2 are both $-N(H)C(O)O-$ may be prepared by a process which comprises reacting a compound of formula (X) as defined above with a compound of formula (XII):



wherein R^x and the remaining variables are all as defined above.

The intermediates of formula (XII) may be prepared by treating a compound of formula (XIII):



(XIII)

wherein the variables are all as defined above; with, for example, phosgene, 4-nitrophenyl chloroformate or *N,N'*-disuccinimidyl carbonate.

Where R^2 is C_{1-4} alkyl, attachment of the R^2 moiety may be effected by conventional *N*-alkylation procedures.

The compounds of formula (II) wherein P^1 and P^2 are identical and A^1 and A^2 are both -NHCONH- may be prepared by a process which comprises reacting a compound of formula $P^1-N=C=O$ with a compound of formula (V) as defined above.

The requisite intermediate of formula $P^1-N=C=O$ may, for example, be prepared by treating a compound of formula (X) as defined above with phosgene.

Where they are not commercially available, the compounds of formula (IV), (VI), (VII), (IX), (X), (XI) and (XIII) may be prepared by methods analogous to those described in the accompanying Examples, or by standard methods well known from the art.

Where a mixture of products is obtained from any of the processes described above for the preparation of compounds according to the invention, the desired product can be separated therefrom at an appropriate stage by conventional methods such as gel permeation chromatography; cation or anion exchange; preparative HPLC; or column chromatography utilising, for example, silica and/or alumina in conjunction with an appropriate solvent system.

During any of the above synthetic sequences it may be necessary and/or desirable to protect sensitive or reactive groups on any of the molecules concerned. This may be

achieved by means of conventional protecting groups, such as those described in *Protective Groups in Organic Chemistry*, ed. J.F.W. McOmie, Plenum Press, 1973; and T.W. Greene & P.G.M. Wuts, *Protective Groups in Organic Synthesis*, John Wiley & Sons, 3rd edition, 1999. The protecting groups may be removed at any convenient subsequent stage utilising methods known from the art.

The following non-limiting Examples illustrate the invention.

Intermediate 1

(4-Maleimidyl-butyl)-carbamic acid tert-butyl ester

To a solution of N-BOC-1,4-diaminobutane (8.324g, 0.044mol) in dry toluene (50ml) was added maleic anhydride (4.336g, 0.044mol) and the solution heated at reflux under Dean-Stark conditions for two days. The solvent was removed and the residue purified by silica column chromatography eluting with 25-40% ethyl acetate in hexane to give the title compound, 1.461g, 12%, as a white solid.

m/z (LCMS ES+, 70V) 291 (MH⁺).

δ_H (CDCl₃) 6.63 (2H, s), 4.46 (1H, br), 3.48 (2H, t, J7.1Hz), 3.08 (2H, m), 1.56 (2H, p, J7.4Hz), 1.41 (2H, m), 1.38 (9H, s).

Intermediate 2

meso-2,3-Bis-tert-butoxycarbonylamino-succinic acid

To a solution of *meso*-2,3-diamino succinic acid (1.96g, 0.013mol) and triethylamine (5.36g, 0.053mol) in water (50ml) was added a solution of di-*tert*-butyl dicarbonate (6.35g, 0.029mol) in dioxane (30ml) over a period of 20min. After 2 hours the solvent was reduced to 10ml, diluted with water to 50ml and washed with dichloromethane (4x30ml). The solution was then acidified to pH 1-2 with 2M HCl and extracted into ethyl acetate (5x50ml). The ethyl acetate solution was dried over magnesium sulphate and the solvent removed to yield the title compound, 4.345g, 94%, as a colourless glass.

m/z (LCMS ES+, 70V) 371 (MNa⁺).

δ_H (d₆-DMSO, 370K) 6.03 (2H, br), 4.49 (2H, s), 1.42 (18H, s).

Intermediate 3**{*tert*-Butoxycarbonyl-[2-(*tert*-butoxycarbonyl-carboxymethyl-amino)-ethyl]-amino}-acetic acid**

- To a stirred suspension of ethylenediamine-N,N'-diacetic acid (1.00g, 5.68mmol) in methanol (150ml) was added triethylamine (2.30g, 22.7mmol) followed by di-*tert*-butyl dicarbonate (2.48g, 11.4mmol). The suspension was heated at reflux for 10 minutes until the majority of solids had dissolved then allowed to cool to ambient temperature and stirred for 2h. The suspension was filtered, the solvent removed, the residue dissolved in water (40ml) and the pH raised to approximately 8-9 with triethylamine. The solution was washed with dichloromethane (5x40ml), acidified with 1M HCl to a pH of approximately 1 and extracted with DCM (10x40ml) followed by ethyl acetate (10x40ml). The ethyl acetate and dichloromethane fractions were dried over magnesium sulphate, combined and the solvent removed to yield the desired material, 1.558g, 74%, as a white solid.
- m/z (LCMS ES+, 70V) 399 (MNa⁺).
- δ_H (d6-DMSO, 380K) 3.86 (4H, s), 3.37 (4H, s), 1.42 (18H, s).

Intermediate 4**3-(*tert*-Butoxycarbonyl-{2-[*tert*-butoxycarbonyl-(2-carboxy-ethyl)-amino]-ethyl}-amino)-propionic acid**

- To ethylene diamine-N,N'-dipropionic acid di-hydrochloride salt (2.00g, 7.2mmol) in water (50ml) and triethylamine (4.38g, 43mmol) was added di-*tert* butyl dicarbonate (3.307g, 15mmol) in dioxane (30ml) over a period of 10 minutes. The reaction was left overnight at ambient temperature, the dioxane removed under reduced pressure and the remaining aqueous solution washed with dichloromethane (4x50ml). The aqueous layer was acidified with concentrated HCl to a pH of approximately 1, and the resulting white precipitate extracted into ethyl acetate (5x60ml). The ethyl acetate solution was dried over magnesium sulphate and the solvent removed to yield the product, 2.68g, 92%, as a white solid.
- m/z (LCMS ES+, 70V) 472 (MNa⁺).
- δ_H (d6-DMSO, 380K) 3.41 (4H, t, J7.2Hz), 3.31 (4H, s), 2.46 (4H, t, J7.2Hz) 1.44 (18H, s).

Intermediate 5**2,3-Bis-*tert*-butoxycarbonylamino-succinic acid bis-succinimidyl ester**

To Intermediate 2 (1.500g, 4.31mmol) in dichloromethane (20ml) were added N-hydroxy succinimide (1.240g, 10.78mmol) and EDC (2.066g, 10.78mmol). After
5 overnight reaction the solution was diluted to 50ml with dichloromethane, washed with water (3x30ml), dried over magnesium sulphate and the solvent removed to give a white solid residue. The residue was purified by silica column chromatography eluting with 50-65% ethyl acetate in hexane to yield the desired di-NHS ester, 277mg, 12%, as a white solid.

10 m/z (LCMS ES⁺, 70V) 565 (MNa⁺).

δ_H (CDCl₃-rotamers) 6.47, 6.17, 6.10 (2H, 3xbr), 5.35, 5.25, 5.10 (2H, 3xbr), 2.82 (8H, s), 1.44, 1.42 (18H, 2xs).

Intermediate 6

15 **{*tert*-Butoxycarbonyl-[2-(*tert*-butoxycarbonyl-carboxymethyl-amino)-ethyl]-amino}-acetic acid bis-succinimidyl ester**

To a suspension of Intermediate 3 (200mg, 0.53mmol) in dichloromethane (4ml) was added triethylamine (269mg, 2.66mmol) and once a clear solution was obtained, N-hydroxy succinimide (153mg, 1.33mmol) followed by EDC (255mg, 1.33mmol) were
20 added. LCMS of the reaction at 45min and 5h showed it to have stopped before completion. An extra 1.5 equivalents of both N-hydroxy succinimide and EDC were added in DCM (3ml) and the reaction left overnight during which time a poorly soluble white solid formed. The reaction mix was diluted to 40ml with dichloromethane, the solution/suspension washed with 0.1M HCl (6x50ml), dried over magnesium sulphate
25 and the solvent removed. The resulting white solid residue was purified by silica column chromatography eluting with 70% ethyl acetate in hexane to yield the desired di-NHS ester, 38mg, 13%, as a poorly soluble white solid.

m/z (LCMS ES⁺, 70V) 593 (MNa⁺).

δ_H (d6-DMSO, 380K) 4.36 (4H, s), 3.46 (4H, s), 2.84 (8H, s), 1.45 (18H, s).

Intermediate 7**3-(*tert*-Butoxycarbonyl-{2-[*tert*-butoxycarbonyl-(2-carboxy-ethyl)-amino]-ethyl}-amino)-propionic acid bis succinimidyl ester**

- 5 To Intermediate 4 (1.00g, 2.48mmol) in dichloromethane (40ml) were added triethylamine (751mg, 7.43mmol), N-hydroxy succinimide (712mg, 6.19mmol) and EDC (1.186g, 6.19mmol). After 2h20min a further 474mg of EDC was added and the reaction left overnight at ambient temperature. The solvent was removed and the residue purified by silica column chromatography, eluting 70% ethyl acetate in hexane to yield the
- 10 product, 773mg, 52%, as a white solid.
m/z (LCMS ES+, 70V) 621 (MNa⁺).
 δ_H (d6-DMSO, 380K) 3.54 (4H, t, J7.0Hz), 3.37 (4H, s), 2.91 (4H, t, J7.0Hz), 2.83 (8H, s), 1.46 (18H, s).

15 **Intermediate 8**

2,3-Bis-*tert*-butoxycarbonylamino-N,N'-bis-(4-maleimidyl-butyl)-succinamide

- To Intermediate 1 (300mg, 1.120mmol) was added 1:1 trifluoroacetic acid: dichloromethane (8ml). After 30 minutes the solvent was removed, the residue dissolved in dichloromethane and Intermediate 5 (276mg, 0.509mmol) added, followed
- 20 immediately by triethylamine (258mg, 2.546mmol). After 30 minutes PS-TsCl scavenger resin (0.5g, 1.44mmol/g) was added, stirred for 1 hour and filtered off. The solvent was removed and the residue purified by silica column chromatography eluting with 75-100% ethyl acetate in hexane to give the desired material, 115mg, 35%, as a white solid.
m/z (LCMS ES+, 70V) 649 (MH⁺).
- 25 δ_H (CDCl₃) 6.64 (2H, br), 6.62 (4H, s), 6.28 (2H, br), 4.30 (2H, br), 3.46 (4H, t, J6.9Hz), 3.17 (4H, m), 1.57-1.40 (8H, m), 1.39 (18H, s).

Intermediate 9

- (2-{*tert*-Butoxycarbonyl-[2-(methoxy-polyethoxy)-ethylcarbamoyl]-methyl}-amino)-ethyl)-[2-(methoxy-polyethoxy)-ethylcarbamoyl]-methyl]-carbamic acid *tert*-butyl ester**
- 30

To a solution of Intermediate 6 (5.7mg, 0.010mmol) in dichloromethane (5ml) were added 20K methoxy-PEG-amine (500mg, 0.025mmol) (purchased from Rapp

Polymere) and triethylamine (5.1mg, 0.050mmol). The reaction was left overnight, diluted with dichloromethane to 25ml and stirred for 3 days with MP-Tosic acid scavenger resin (3.0g, 1.43mmol/g). The resin was filtered off, the solution diluted to 50ml with dichloromethane and washed with 0.1M HCl (4x30ml). The solution was
 5 dried over magnesium sulphate and the solvent removed to give the 40K product, 370mg, 92%, as a waxy white solid.
 δ_{H} (CDCl₃) 4.1-3.3 (~3600H, brm), 3.30 (6H, s), 1.42 (18H, br).

Intermediate 10

10 (2-{*tert*-Butoxycarbonyl-[2-(2-(methoxy-polyethoxy)-ethylcarbamoyl)-ethyl]-amino}-ethyl)-[2-(2-(methoxy-polyethoxy)-ethylcarbamoyl)-ethyl]-carbamic acid *tert*-butyl ester

To a solution of Intermediate 7 (11.1mg, 0.019mmol) in dichloromethane (10ml) were added 20K methoxy-PEG-amine (1.00g, 0.05mmol) (purchased from Rapp
 15 Polymere) and triethylamine (9.4mg, 0.093mmol). The reaction was left overnight, diluted with dichloromethane to 50ml and stirred for 3 days with MP-Tosic acid scavenger resin (6.0g, 1.43mmol/g). The resin was filtered off, the solution washed with 0.1M HCl (4x50ml), dried over magnesium sulphate and the solvent removed to quantitatively yield the 40K product as a waxy white solid.
 20 δ_{H} (CDCl₃) 4.5-3.0 (~3600H, brm), 3.30 (6H, s), 2.4 (4H, br), 1.37 (18H, brs).

Intermediate 11

2,3-Diamino-N,N'-bis-[4-maleimidyl-butyl]-succinamide bis TFA salt

Intermediate 8 (4.0mg, 0.0062mmol) was dissolved in dichloromethane (1ml) and
 25 trifluoroacetic acid (1ml) added. After half an hour the solvent was removed and the residue used crude in the synthesis of Example 1.

m/z (LCMS ES⁺, 70V) 449 (MH⁺).

δ_{H} (d₆-DMSO) 6.93 (4H, s), 4.14 (2H, s), 3.32 (4H, t, J6.8Hz), 3.06, 2.96 (4H, 2xm), 1.42 (4H, m), 1.29 (4H, m).

Intermediate 12**N-(2-(Methoxy-polyethoxy)-ethyl)-2-(2-[(2-(methoxy-polyethoxy)-ethylcarbamoyl)-methyl]-amino)-ethylamino)-acetamide di-hydrochloride**

Intermediate 9 (approx 400mg) was dissolved in dichloromethane (4ml), and
5 trifluoroacetic acid (4ml) added. After half an hour the solvent was removed, the residue dissolved in dichloromethane (100ml), washed with 0.1M HCl (3x100ml), dried over magnesium sulphate and the solvent removed to yield the salt (386mg) as an off-white solid.

δ_H (CDCl₃) 4.2-3.0 (~3600H, brm), 3.31 (6H, s).

10

Intermediate 13**N-(2-(Methoxy-polyethoxy)-ethyl)-3-{2-[2-(2-(methoxy-polyethoxy)-ethylcarbamoyl)-ethylamino]-ethylamino}-propionamide di hydrochloride salt**

Intermediate 10 (460mg) was dissolved in dichloromethane (5ml), and
15 trifluoroacetic acid (5ml) added. After half an hour the solvent was removed, the residue dissolved in dichloromethane (60ml), washed with 0.1M HCl (4x60ml), dried over magnesium sulphate and the solvent removed to yield the salt (460mg) as a white solid.
 δ_H (CDCl₃) 7.20 (2H, br), 4.0-3.0 (~3600H, brm), 3.31 (6H, s), (remaining signals obscured by broad H₂O signal).

20

Example 1**N,N'-Bis-[4-maleimidylbutyl]-2,3-bis-(3-(methoxy-polyethoxy)-propionylamino)-succinamide**

To Intermediate 11 (4.0mg, 6.2micromol) in dichloromethane (8ml) were added
25 M-PEG-SPA from Nektar (formerly Shearwater) (393mg, MW22K) followed by triethylamine (13mg, 123 μ mol). The reaction was stirred at ambient temperature for 3 days followed by 2 days at gentle reflux. To the reaction was added MP-TsCl resin (0.25g, 1.44mmol/g), the reaction gently stirred for 2 hours and the resin filtered off. The reaction was diluted to 50ml with dichloromethane, washed with 0.1M HCl (3x30ml),
30 dried over magnesium sulphate and the solvent removed. ¹H NMR showed the presence of unreacted M-PEG-SPA so the residue was dissolved in distilled water (10ml) and left overnight to hydrolyse the ester. The aqueous solution was washed with diethyl ether (2x50ml), extracted into dichloromethane (5x50ml), dried over magnesium sulphate and

the solvent removed to yield a white solid containing a mixture of mPEG-propionic acid, the mono-PEGylated species and the desired di-PEGylated species.

δ_{H} (CDCl_3) 7.95 (2H, br), 7.55 (2H, br), 6.63 (4H, s), 4.89 (2H, br), 4.1-3.0 (~4000H, brn), 2.52 (4H, t), 1.58 (4H, m), 1.38 (4H, m).

5

Example 2

3-Maleimidyl-N-(2-{[3-maleimidyl-propionyl]-[2-(methoxy-polyethoxy)-ethylcarbamoyl]-methyl}-amino)-ethyl)-N-[2-(methoxy-polyethoxy)-ethylcarbamoyl]-methyl)-propionamide

10 Intermediate 12 (386mg, 0.0097mmol) was dissolved in dichloromethane (7ml) and to it added maleimido propionic acid N-hydroxysuccinimide ester (6.4mg, 0.024 mmol) followed by triethylamine (39mg, 0.386mmol). After overnight reaction the solvent was removed, the residue dissolved in dichloromethane (6ml) and half dropped into rapidly stirring diethyl ether (80ml) to give a white precipitate. This was filtered off
15 under an atmosphere of nitrogen, dissolved in dichloromethane and the solvent removed to give a material with only partial maleimide addition. An 80mg batch of this material was retreated with 10 equivalents of maleimido propionic acid N-hydroxysuccinimide ester and 40 equivalents of triethylamine in dichloromethane (2ml) for 3 days, giving, after precipitation from diethyl ether, 80mg of a substantially unchanged product. This
20 material was then treated with 10 equivalents of maleimido propionyl chloride (from maleimido propionic acid treated with 1:1 oxalyl chloride:dichloromethane (4ml) for 3 hours at ambient temperature) and 40 equivalents of triethylamine, giving, after precipitation from diethyl ether, material determined by ^1H NMR to consist of roughly a 1:1 mixture of the desired di-maleimide and the mono-maleimide species.
25 δ_{H} (CDCl_3) 6.65 (4H, m), 4.2-3.2 (~3600H, brn), 3.31 (6H, s), 2.57 (4H, m).

Example 3

3-Maleimidyl-N-(2-{[3-(maleimidyl)-propionyl]-[2-(2-(methoxy-polyethoxy)-ethylcarbamoyl)-ethyl]-amino}-ethyl)-N-[2-(2-(methoxy-polyethoxy)-ethylcarbamoyl)-ethyl)-propionamide

30

To maleimido propionic acid in dichloromethane (2ml) was added oxalyl chloride (2ml) and after 4.5 hours the solvent thoroughly removed under reduced pressure. To this crude acid chloride was added Intermediate 13 (116mg, 2.9 μ mol) in dichloromethane

(2ml) followed by triethylamine (12mg, 116 μ mol). After three days the solvent was removed, the residue redissolved in dichloromethane (3ml) and added slowly to rapidly stirred diethyl ether (100ml). The resulting white precipitate was filtered off, washed with diethyl ether, redissolved in dichloromethane and filtered. The solvent was then removed to yield the product in quantitative yield as a white, waxy solid.

δ_{H} (CDCl₃) 6.81 (1H, t), 6.78 (1H, t), 6.69, 6.68 (4H, 2xs), 4.0-3.2 (~3600H, brm), 3.35 (6H, s), 2.72 (4H, m), (remaining signals obscured by broad H₂O signal).

Example 4

DiFab' Conjugate of Example 1 Product

5ml of an engineered Fab' containing a single hinge thiol (see for example US 5,677,425; WO 98/25971), at 20mg/ml in 0.1M phosphate, 2mM EDTA, pH6, was reduced with a 20-fold molar excess of 2-mercaptoethylamine for 1 hour at ambient temperature. The reductant was then removed by gel filtration on five PD10 columns in parallel equilibrated in 0.1M phosphate, 2mM EDTA, pH6. The concentration of the pooled reduced Fab' was determined by A280 (11.8mg/ml) and then 40mg of reduced Fab' was conjugated to 20mg of Example 1 (stock solution at 50mg/ml in 0.1M phosphate, 2mM EDTA, pH6) for 16 hours at ambient temperature. The extent of conjugation was assessed by SDS-PAGE and size-exclusion HPLC. To 3.33ml of the Fab':PEG conjugation reaction was added 6.67ml of distilled water and 100 μ l of 1M acetic acid, and 10ml of this was loaded onto a 4ml SP-Sepharose HP C10 column (GE Healthcare) equilibrated in 50mM acetate, pH4.5, at 2ml/min. The column was eluted with a 16ml linear gradient of 0-250mM NaCl in 50mM acetate, pH4.5. 2ml fractions were collected and those containing PEG-DiFab' as assessed by SDS-PAGE were pooled.

The pooled fractions were concentrated with 10000 MWCO spin cartridges to 310 μ l. 300 μ l of this was loaded onto a Superose 6 HR10/30 gel filtration column (GE Healthcare) equilibrated in 50mM acetate, 125mM NaCl, pH5.5, at 0.5ml/min. The Superose 6 column was eluted with an isocratic gradient of 50mM acetate, 125mM NaCl, pH5.5, at 0.5ml/min. 0.5ml fractions were collected and those containing only PEG-DiFab' (#B7-B5) as assessed by SDS-PAGE were pooled. The pooled fractions were concentrated with 10000 MWCO spin cartridges to 100 μ l. The concentration of the PEG-DiFab' was determined by A280 and the purity was assessed by reducing and non-reducing SDS-PAGE.

Example 5**DiFab' Conjugate of Example 2 Product**

4ml of an engineered Fab' containing a single hinge thiol (see for example
5 US 5,677,425; WO 98/25971) at 20mg/ml in 0.1M phosphate, 2mM EDTA, pH6, was
reduced with a 20-fold molar excess of 2-mercaptoethylamine for 1 hour at ambient
temperature. The reductant was then removed by gel filtration on four PD10 columns in
parallel equilibrated in 0.1M phosphate, 2mM EDTA, pH6. The concentration of the
pooled reduced Fab' was determined by A280 (12.0mg/ml) and then 22.7mg of reduced
10 Fab' was conjugated to 4.8mg of Example 2 for 16 hours at ambient temperature. The
extent of conjugation was assessed by reducing and non-reducing SDS-PAGE and size-
exclusion HPLC. Conjugate purification and analysis as for Example 4.

Example 6**DiFab' Conjugate of Example 3 Product**

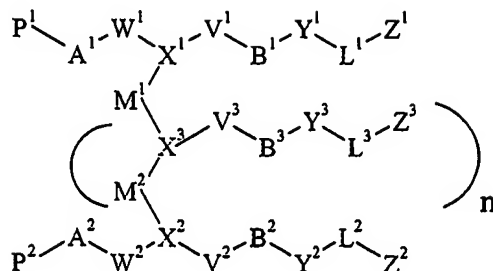
4 ml of an engineered Fab' containing a single hinge thiol (see for example
US 5,677,425; WO 98/25971) at 20mg/ml in 0.1M phosphate, 2mM EDTA, pH6, was
reduced with a 20-fold molar excess of 2-mercaptoethylamine for 1 hour at ambient
temperature. The reductant was then removed by gel filtration on four PD10 columns in
20 parallel equilibrated in 0.1M phosphate, 2mM EDTA, pH6. The concentration of the
pooled reduced Fab' was determined by A280 (14.6mg/ml) and then in duplicate 14.2mg
of reduced Fab' was conjugated to 4mg of Example 3 (stock solution at 20mg/ml in
distilled water) for 16 hours at ambient temperature. The extent of conjugation was
assessed by SDS-PAGE and size-exclusion HPLC. The duplicate Fab':PEG conjugation
25 reactions were pooled and to them was added 7.56ml of distilled water and 100µl of 1M
acetic acid. 9.7ml of this was loaded onto a 4ml SP-Sepharose HP C10 column (GE
Healthcare) equilibrated in 50mM acetate, pH4.5, at 2ml/min. The column was eluted
with a 16ml linear gradient of 0-250mM NaCl in 50mM acetate, pH4.5. 2ml fractions
were collected and those containing PEG-DiFab' as assessed by SDS-PAGE were pooled.
30 The pooled fractions were concentrated with 10000 MWCO spin cartridges to 260µl.
200µl of this was loaded onto a Superose 6 HR10/30 gel filtration column (GE
Healthcare) equilibrated in 50mM acetate, 125mM NaCl, pH5.5, at 0.5ml/min. The
Superose 6 column was eluted with an isocratic gradient of 50mM acetate, 125mM NaCl,

pH5.5 at 0.5ml/min. 0.5ml fractions were collected and those containing only PEG-DiFab' as assessed by SDS-PAGE were pooled. The pooled fractions were concentrated with 10000 MWCO spin cartridges to 570µl. The concentration of the PEG-DiFab' was determined by A280 (3.5mg/ml) and the purity was assessed by

5 reducing and non-reducing SDS-PAGE, endotoxin assay and size-exclusion HPLC.

Claims:

1. A compound of formula (I):



(I)

wherein

P^1 and P^2 independently represent a polymer residue;

Z^1 , Z^2 and Z^3 independently represent the residue of a biologically active moiety;

X^1 , X^2 and X^3 independently represent CR^1 or N;

A^1 and A^2 independently represent $-CONH-$, $-NHCO-$, $-OC(O)N(R^2)-$, $-N(R^2)C(O)O-$ or $-NHCONH-$;

B^1 , B^2 and B^3 independently represent $-CONH-$ or $-CO-$;

V^1 and V^2 independently represent a covalent bond or $-(CH_2)_v-$;

W^1 and W^2 independently represent a covalent bond or $-(CH_2)_w-$;

Y^1 , Y^2 and Y^3 independently represent $-(CH_2)_y-$;

L^1 , L^2 and L^3 independently represent a spacer group;

M^1 and M^2 independently represent a covalent bond or $-(CH_2)_m-$;

R^1 represents hydrogen or C_{1-4} alkyl;

R^2 represents hydrogen or C_{1-4} alkyl;

n is zero, 1 or 2;

v is 1, 2, 3 or 4;

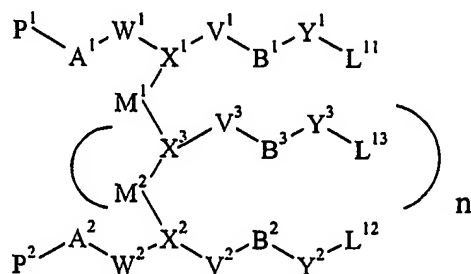
w is 1, 2, 3 or 4;

y is 1, 2, 3, 4, 5 or 6; and

m is 1, 2 or 3.

2. A compound as claimed in claim 1 wherein Z^1 , Z^2 and Z^3 independently represent the residue of a whole antibody or a functionally active fragment or derivative thereof.

5 3. A compound of formula (II):



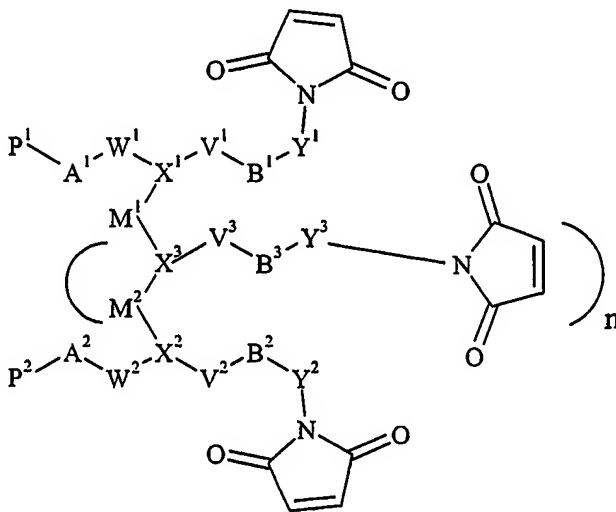
(II)

wherein

10 L^{11} , L^{12} and L^{13} represent groups capable of attaching the residue Z^1 , Z^2 and Z^3 respectively, or capable of being converted into such groups; and
 Z^1 , Z^2 , Z^3 and each of the other variables is as defined in claim 1.

4. A compound as claimed in claim 3 represented by formula (III):

15



(III)

wherein each of the variables is as defined in claim 1.

5. A compound as claimed in any one of the preceding claims wherein P^1 and P^2 independently represent a polyethylene glycol (PEG) residue.

5

6. A compound as claimed in any one of the preceding claims wherein R^1 is hydrogen.

7. A compound as claimed in any one of the preceding claims wherein n is zero.

10

8. A compound of formula (I) as defined in claim 1 as herein specifically disclosed in Example 4, 5 or 6.

9. A compound of formula (III) as defined in claim 4 as herein specifically disclosed in Example 1, 2 or 3.

15

10. A pharmaceutical composition comprising a compound of formula (I) as defined in claim 1 in association with one or more pharmaceutically acceptable carriers, excipients or diluents.

20

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FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO,
SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN,
GQ, GW, ML, MR, NE, SN, TD, TG).

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3 November 2005

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: **BRANCHED POLYETHYLENE GLYCOL FOR LINKING POLYMER RESIDUES TO ANTIBODIES**

(57) Abstract: Branched molecular scaffolds are provided which are capable of linking two polymer residues (derived, for example, from polyethylene glycol) to two, three or four residues derived from biologically active molecules (e.g. from whole antibodies or from functionally active fragments or derivatives thereof), the latter being attached to the scaffold by means of hydrolytically stable linkages.



WO 2005/061005 A3

INTERNATIONAL SEARCH REPORT

PCT/GB2004/005246

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, CHEM ABS Data, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2002/009426 A1 (GREENWALD RICHARD B ET AL) 24 January 2002 (2002-01-24) page 13, column 1, paragraph 2-4; figures 1,4,7,8 page 14, column 1, paragraph 4	1-3,5-7, 10
X	HURWITZ E ET AL: "Inhibition of tumor growth by poly(ethylene glycol) derivatives of anti-ErbB2 antibodies" CANCER IMMUNOLOGY AND IMMUNOTHERAPY, BERLIN, DE, vol. 49, no. 4-5, July 2000 (2000-07), pages 226-234, XP002254077 ISSN: 0340-7004 page 228, column 1; figure 1 -/-	1-3,5-7, 10

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

31 August 2005

Date of mailing of the international search report

07/09/2005

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Gonzalez Ramon, N

INTERNATIONAL SEARCH REPORT

PCT/GB2004/005246

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/031475 A (CELLTECH R & D LIMITED; POPPLEWELL, ANDREW, GEORGE; TICKLE, SIMON, PET) 17 April 2003 (2003-04-17) page 10, lines 15-25; claims 24-27,36; figure 6 page 24, line 30 - page 25, line 5	1-7,10
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Y	CHAPMAN A P ET AL: "THERAPEUTIC ANTIBODY FRAGMENTS WITH PROLONGED IN VIVO HALF-LIVES" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 17, no. 8, August 1999 (1999-08), pages 780-783, XP001199462 ISSN: 1087-0156 figure 2	1-7,10
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E	EP 1 496 076 A (BEIJING JIANKAI TECHNOLOGY CO., LTD) 12 January 2005 (2005-01-12) claims 6,7,13; example 7	3-7,10
T	NATARAJAN A. ET AL: "Characterization of site specific ScFv PEGylation for tumor targeting pharmaceuticals" BIOCONJUGATE CHEM., vol. 16, no. 1, 2005, pages 113-121, XP008048580 abstract; figure 1 page 117, column 2 page 119, column 1, paragraph 2	1-7,10

-/-

INTERNATIONAL SEARCH REPORT

PCT/GB2004/005246

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	MANTOVANI G. ET AL: "Design and synthesis of N-maleimido-functionalized hydrophlic polymers via copper mediated living radical polymerization: a suitable alternative to PEGylation chemistry" J. AM. CHEM. SOC., vol. 127, no. 9, 2005, pages 2966-2973, XP008048582 abstract; figures 3-5 see conclusions page 2971, column 2	1,3-6,10
X,P	US 2004/225097 A1 (NHO KWANG ET AL) 11 November 2004 (2004-11-11) page 3, column 1; examples 3,4	3-7,10

INTERNATIONAL SEARCH REPORT

PCT/GB2004/005246

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 8,9
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 8,9

Claims 8, 9 do not define subject matter in terms of technical features (Art 6 PCT)

A claim should be understandable without reference to the description: "as herein specifically disclosed in the examples" cannot be accepted under Art. 6 PCT and Rule 6.2(a).

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

PCT/GB2004/005246

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